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ASSOCIATED LEUKOCYTE RESPONSES IN THE LETHAL ASPECTS OF 'E. COL--ETC(U)
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ASSOCIATED LEUKOCYTE RESPONSES IN THE LETHAL ASPECTS OF E. COLI SHOCK

L. B. Hinshaw, B. K. Beller, L. T. Archer, and G. L. White

Prepared for Publication

in

Proceedings of the Society for Experimental
Biology and Medicine

University of Oklahoma Health Sciences Center
Departments of Physiology and Biophysics and Research Surgery
Oklahoma City, Oklahoma

15 April 1977

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Dogs administered lethal injections of E. coli endotoxin or E. coli organisms develop systemic hypotension, hypoglycemia and hepatosplanchnic dysfunction (1-4). Progressively decreasing blood glucose levels after endotoxin or E. coli administration are due in large part to depressed hepatic function, particularly gluconeogenesis (4-7). Accelerated glucose uptake has been reported following in vitro incubation of either endotoxin or live E. coli organisms in blood, and white blood cell (WBC) phagocytic activity has been implicated as the primary responsible factor (2). Increased phagocytic activity of the blood after endotoxin (8) has been traced to the buffy coat (9) and the leukocyte (10). Recent reports have shown circulating neutrophils to be of major importance in the clearance of bacterial organisms (11) or endotoxin (12) from the blood, while others have described beneficial effects of transfused WBC's in patients and animals in septic shock (13,14).

The purpose of the present study was to explore the responses of canine blood to the separate effects of E. coli organisms and E. coli endotoxin, particularly emphasizing the role of the WBC in the uptake of glucose in vitro and its possible relationship to survival in vivo.

Materials and methods. In vivo experiments were carried out on twelve awake adult mongrel dogs during a 4-day period. On the fourth day, venous blood was drawn from each animal and additionally studied in the in vitro state. Animals, selected for robust health and absence of heart worms, were treated for intestinal parasites and conditioned in the animal facility for 3-6 weeks prior to use. Dogs with initial WBC counts between 7,000 and 20,000/mm³ and hematocrits exceeding 37% were utilized in the experiments.

In vivo studies. Unanesthetized, gently restrained animals were divided into paired control and experimental groups which were studied simultaneously. The experimental group received sublethal doses of endotoxin (Difco, Detroit); 1/1,000 LD₁₀₀ on days 1 and 2 (0.003 mg/kg body weight), 1xLD₁₀₀ on day 3 (3 mg/kg), followed by 2xLD₁₀₀ live E. coli on day 4 (2.5×10^{10} organisms/kg). The control group received equal volumes of saline on days 1, 2 and 3, and on day 4 received the identical dose of E. coli organisms as in the experimental group. The LD₁₀₀ of E. coli endotoxin and E. coli organisms was previously established in this laboratory. Animals living 6 days following injection of E. coli were considered permanent survivors.

In vitro studies. An in vitro system served as a test device to assay responses of the blood to E. coli endotoxin and E. coli organisms in the absence of the organs of gluconeogenesis and with the prevention of the cell migration which occurs in vivo. Accelerated uptake of glucose by the blood, ascribed to increased metabolic activity of white blood cells, was described in an earlier study (2). Blood for in vitro studies was drawn intravenously from the twelve awake dogs on the fourth day prior to their receiving E. coli injections and incubated as previously reported (2). Three tubes of blood obtained from each control (saline-pretreated) and experimental (sublethal endotoxin-pretreated) animal were studied in vitro following separate additions of E. coli or endotoxin, at LD₁₀₀ doses, or saline.

WBC counts were measured with an automatic particle counter (Coulter ZF; Hialeah, Florida) and the differential WBC by microscopic examination of blood stained with Wrights stain. Blood

glucose concentrations were determined with a Beckman Glucose Analyzer (Beckman Instruments; Fullerton, Calif.) possessing an accuracy of ± 3 mg%. Venous blood samples for in vivo studies were placed in vacutainers containing ethylenediamine-tetraacetic acid (EDTA; Beckton-Dickinson). Blood samples for in vitro studies in 10 ml volumes were anticoagulated with heparin (0.1 ml; 20,000 units/ml) and incubated in a water bath at 37° - 38°C for 3-6 hours. Results from all experiments were analyzed using the t test for paired or unpaired data.

Results. Figure 1 presents in vivo WBC data obtained from animals receiving single sublethal injections of endotoxin on days 1, 2 and 3, and superlethal administrations of E. coli organisms on day 4. Daily values were obtained prior to injections of endotoxin in the experimental group or saline in the control, and values on days 2, 3 and 4 are seen to reflect the effects of the previous injections. Significant leukocytosis ($p \leq 0.05$) is observed in the experimental group on days 2, 3 and 4, which is accounted for primarily by elevations in blood concentrations of mature and immature neutrophils, while insignificant changes occur in the lymphocyte and monocyte populations. Following injections of $2 \times \text{LD}_{100}$ E. coli organisms on the fourth day, leukopenia and neutropenia were observed in experimental and control groups for 2 hours ($p < 0.01$), cell counts showing recovery to near pre-injection values within 6 hours. Mean hourly WBC concentrations in the experimental group during days 1-3 are not shown but by the first hour after sublethal endotoxin administration were lower than the control group ($p = 0.001$) and elevated above it within 6 hours ($p < 0.05$).

All dogs pretreated with sublethal endotoxin survived following superlethal E. coli administration while every animal pretreated with saline died within 9 hours after E. coli injection, following massive intestinal bloody diarrhea and a protracted moribund condition.

In vitro experiments were carried out to determine the effects of E. coli endotoxin or E. coli organisms on glucose concentrations in blood drawn from animals pretreated with sublethal injections of endotoxin or saline as described above. Samples of blood were withdrawn from animals on day 4 immediately prior to administering superlethal doses of E. coli in vivo. Figure 2 illustrates the mean results from three paired experiments (total N=36; i.e., 3 sets of 12 experiments each, including control groups). Endotoxin (LD₁₀₀), E. coli organisms (LD₁₀₀) or saline were added to separate tubes in vitro immediately after zero time and observed for 3-5 hours. Mean glucose concentrations are seen to fall significantly below control values in all experiments ($p < 0.05$). In vitro glucose concentrations in blood obtained from dogs pretreated with endotoxin in vivo were significantly lower than the in vivo saline-pretreated group within 60 minutes and were also lower than the group receiving only saline in vitro ($p = 0.05$). The endotoxin-pretreated blood groups receiving endotoxin and E. coli utilized significantly greater quantities of glucose within 2 hours than the saline groups ($p = 0.05$). The saline-pretreated blood receiving E. coli in vitro revealed similarly low glucose values by 3 hours. Both the in vitro saline control groups and endotoxin group comprised of blood obtained from saline-pretreated animals demonstrated less marked declines in glucose concentrations during the 2-3 hour period. There were no significant

differences between values of the endotoxin-pretreated blood administered endotoxin in vitro and the saline-pretreated blood to which E. coli was added ($p > 0.05$).

It was considered of interest to estimate the rate of glucose uptake per WBC in endotoxin- vs. saline-pretreated blood to which LD₁₀₀ endotoxin was added in vitro. Previously reported work (2) and parallel studies carried out in this laboratory have implicated the white blood cell as the primary component of blood responsible for increased uptake of glucose following addition of endotoxin in vitro. Washed red blood cells, suspended in a glucose-saline solution, did not demonstrate an increased uptake of glucose following addition of endotoxin in vitro (2). On the basis of these earlier observations, calculations were carried out in the present study to estimate the increased rate of glucose uptake per WBC following addition of LD₁₀₀ endotoxin in vitro. This excess quantity of glucose was obtained by subtracting the glucose uptake in blood to which saline alone was added from that to which endotoxin was administered. The excess uptake occurring during the first hour was divided by the average WBC count during the same period, in order to estimate the quantity of excess glucose uptake per WBC. Calculations showed the quantity of excess glucose taken up per activated WBC from blood receiving prior sublethal injections of endotoxin was not different from the nonactivated WBC in vitro (11.7×10^{-9} vs. 7.4×10^{-9} mg glucose/WBC/60 min, respectively) ($p > 0.05$).

Discussion. Progressively developing hypoglycemia in dogs administered endotoxin or E. coli organisms has been documented and found to be associated with systemic hypotension, hepatosplanchnic

pathology and death (1,3). The cause of hypoglycemia has been the subject of much recent research in endotoxin or septic shock. Impaired glucose production as a result of depressed hepatic function has been suggested as a primary factor in the development of hypoglycemia because of adverse effects on gluconeogenesis (4-7). A recent publication from this laboratory suggests that endotoxin also eliminates the gluconeogenic ability of the kidney in the canine species (15).

Recent studies have documented increased uptake of glucose by the blood after endotoxin which partially accounts for the hypoglycemia of shock (2,15). Results from the present study support these earlier observations and further suggest that the accelerated glucose uptake by the blood after endotoxin is primarily due to the increased activity of circulating white blood cells whose rate of glucose utilization varies directly with their total numbers.

Findings from the present investigation suggest a relationship between numbers of white blood cells, particularly neutrophils, and survivability to superlethal doses of E. coli organisms. Daily sublethal intravenous injections of endotoxin administered during a 3-day period resulted in a marked state of leukocytosis. The cause of the elevated numbers of white blood cells was not determined in the present study; however, it is known that endotoxin administration promotes the entry of new leukocytes from the bone marrow into the circulation (16). Animals receiving superlethal injections of E. coli organisms on the fourth day were completely protected against the pathophysiological and lethal effects of the organisms. It is possible that the significantly increased numbers of white blood cells, initially present on the fourth day and

composed primarily of neutrophils, may have efficiently phagocytosed the injected organisms, thereby preserving hepatic function (7), including gluconeogenesis (6). Additionally, hepatosplanchnic pooling, extravasation and bloody diarrhea may have been prevented by augmented white blood cell phagocytotic activity. The degree of protection seemed remarkable: animals receiving prior sublethal injections of endotoxin were eating and drinking, appeared normal in every respect within 12 hours, and all were healthy survivors at 6 days. On the other hand, all animals pretreated only with saline and challenged on the fourth day with superlethal doses of E. coli uniformly demonstrated the development of massive bloody diarrhea, vomiting and a subsequent moribund state, dying within 9 hours post-injection.

The question of possible "activation" of the WBC, in which each cell becomes more phagocytically active, was not supported by the results of the present study. Enhanced phagocytic activity appeared to be due to the increased numbers of white blood cells, glucose uptake per cell being essentially equal in activated and nonactivated cells. The WBC types accounting for the total increase in numbers in the present study were shown to be the mature and immature neutrophils, cells which have been reported to be particularly active in phagocytosing endotoxin (12) or E. coli (11). Recent studies have documented beneficial effects of transfused white blood cells in animals and patients in septic shock (13,14). Results from the present study suggest a relationship between leukocytosis and survivability in septic shock, lending support to the view that increased numbers of white blood cells by way of transfusion may augment the degree of protection.

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FIGURE LEGENDS

Figure 1. White blood cell concentrations and differential white blood cell responses to superlethal dose of E. coli organisms in dogs following previous sublethal injections of E. coli endotoxin (mean \pm SE; N=6 in each group). The values at the "Control (Days)" time designations are initial measurements recorded prior to injection of endotoxin or saline on days 1, 2 and 3, and E. coli organisms on day 4; therefore, the day 4 control value is actually the initial "control" measurement for the values in the "Hours (Day 4)" time designation. The values from 2-8 hours on day 4 are recorded following intravenous administration of E. coli organisms; 2xLD₁₀₀ (2.5×10^{10} org/kg). The experimental (endotoxin) group received sublethal doses of E. coli endotoxin on days 1 and 2 (1/1,000 LD₁₀₀), on day 3 (LD₁₀₀), and a challenge dose of E. coli organisms on day 4 (2xLD₁₀₀). The control (saline) group received equal volumes of saline on days 1, 2 and 3, and on day 4 received 2xLD₁₀₀ E. coli organisms. P values represent an unpaired comparison between control and experimental groups.

Figure 2. Effects of E. coli organisms (LD₁₀₀) and E. coli endotoxin (LD₁₀₀) on blood glucose concentrations in vitro following previous sublethal injections of E. coli endotoxin in vivo (N=6 in each group, total N=36). Endotoxin, E. coli or saline administered immediately after zero time; LD₁₀₀

endotoxin = 2.5×10^{-2} mg/ml blood; LD₁₀₀ E. coli organisms = 3×10^8 organisms/ml blood. Mean glucose concentrations plotted; p values show statistical significances between groups of blood samples obtained from dogs pretreated with endotoxin and saline (see Figure 1 for pretreatment data in vivo).

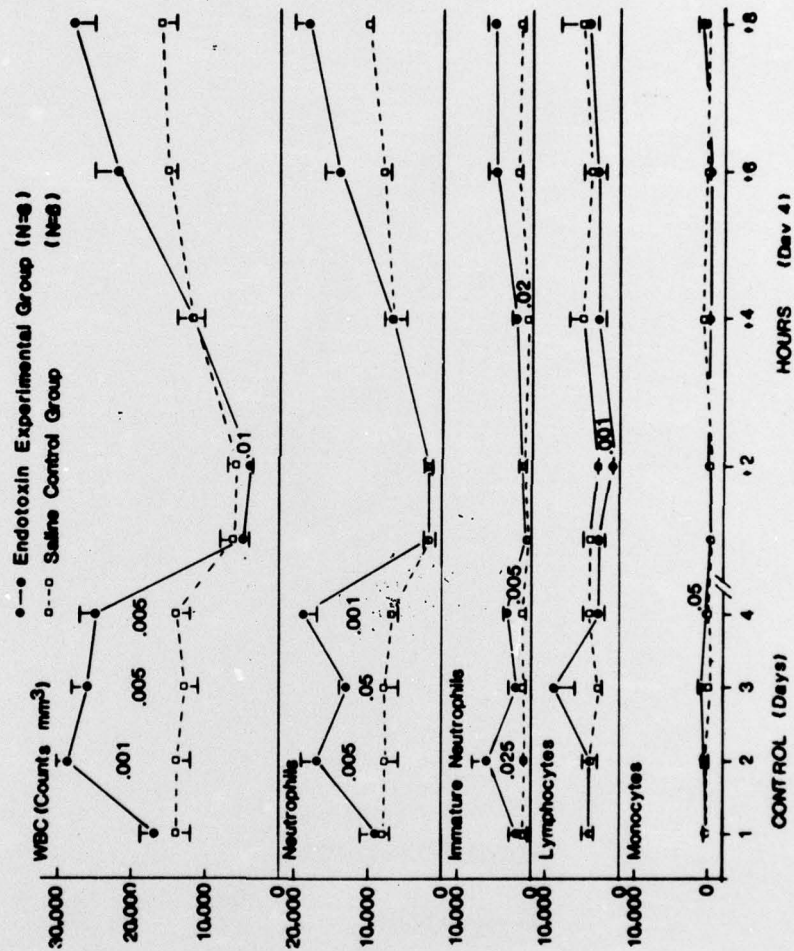


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